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## A STUDY OF THE PNEUMOCOCCUS, WITH ESPECIAL REFERENCE TO THE INULIN TEST.\*

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THE work reported in this paper was carried on under the direction of Drs. William H. Park, and A. W. Williams, and consists of a study of some of the pneumococcus strains isolated at the Research Laboratory during the period between November, 1904, and August, 1905. The system of designating the strains is that used by Drs. Park and Williams in the report made to the Pneumonia Commission,<sup>1</sup> the description of the strains here mentioned being included in that report.

The organisms have been cultivated during the time since isolation upon blood agar, with occasional transfers to serum broth, or calcium broth. The possibility of contamination by similar organisms, during so long a period of cultivation on artificial media, has, of course, to be considered in judging results, but it is not thought that any contamination has taken place; first, because of the care exercised in transferring cultures and watching results; second, because where changes have occurred in the different strains they have been of a similar character, indicating a systematic tendency, and third, because in some cases, where a change has taken place, it has been possible to observe the different stages leading toward it, in a graduated series.

Very few strains are now found to be typical, morphologically. The majority show very small organisms and increased chain formation, namely, a more or less marked approach toward a streptococcus type. With some strains this change seems to be a permanent one, the organisms having a definite streptococcus morphology in all media; with others the change is but temporary, to be followed in later cultures by a return to a characteristic pneumococcus type, and with a few strains, while an occasional atypical culture is seen,

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<sup>1</sup> *Jour. Exper. Med.* 1905, 7, p. 403.

the majority of cultures now show a morphology as typical as when the organisms were first isolated more than a year ago.

Table 1 gives the results of a study of morphology and inulin coagulation made during January, 1906. Sixty-one strains were studied at this time, selected at random from the laboratory stock. Since but one inulin test was made with each strain in this series of experiments, only general conclusions can be drawn as to inulin coagulation from this part of the work, in view of the great irregularities later observed.

TABLE 1.  
SUMMARY OF INULIN TESTS MADE JANUARY, 1906, UPON STRAINS OF PNEUMOCOCCI ISOLATED AT DIFFERENT PERIODS BETWEEN NOVEMBER, 1904, AND MARCH, 1905, COMPARED WITH SIMILAR TESTS MADE SOON AFTER ISOLATION. ALL POSITIVE IN ORIGINAL TESTS.

	COAGULATED NOT LATER THAN IN ORIGINAL TEST		COAGULATED LATER THAN IN ORIGINAL TEST		NOT COAGULATED THIS TIME THOUGH COAGULATED IN ORIGINAL TEST		TOTAL
	Char.	Not Char.	Characteristic	Not Char.	Char.	Not Char.	
PNEUMONIA CASES	8 <sub>1001</sub> *9 <sub>1001</sub> 13 <sub>1001</sub> 46 <sub>1001</sub> 56 <sub>1112</sub> 69 <sub>1001</sub> 82 <sub>1322</sub>	18 <sub>1122</sub> 77 <sub>1001</sub>	15 <sub>1112</sub> 3d later 39 <sub>1001</sub> 2d " *47 <sub>1122</sub> 2d " 57 <sub>1122</sub> 8d " 83 <sub>1001</sub> 10d "	22 <sub>1001</sub> 2d later	4 <sub>1001</sub> 62 <sub>1112</sub> 67 <sub>1001</sub> 72 <sub>1001</sub> 75 <sub>1001</sub> 76 <sub>1001</sub> 98 <sub>1122</sub>	20 <sub>1122</sub> 21 <sub>1122</sub> 36 <sub>1001</sub> *47 <sub>1001</sub> 73 <sub>1001</sub>	
	7	2	5	1	7	5	
	9		6		12		27
NORMAL CASES	N4 <sub>11122</sub> N124 <sub>1001</sub> N135 <sub>1122</sub> N136 <sub>1122</sub>	N106 <sub>1112</sub> N111 <sub>1001</sub> N127 <sub>1112</sub> N113 <sub>1001</sub>	N89 <sub>1122</sub> 8d later N99 <sub>1122</sub> 9d later		N23 <sub>1122</sub> N51 <sub>1122</sub> N52 <sub>1122</sub> N59 <sub>1122</sub> N01 <sub>1222</sub> N100 <sub>1122</sub> N109 <sub>1112</sub> N130 <sub>1112</sub>	*N10 <sub>1112</sub> N11 <sub>1122</sub> N43 <sub>1122</sub> N45 <sub>1001</sub> N107 <sub>1112</sub>	
	4	4	2	0	8	5	
	8		2		13		23
MISCELLANEOUS CASES	C & D <sub>41001</sub> Q5 <sub>1122</sub>	C23 <sub>1112</sub> Mc1 <sub>1122</sub>	C5 <sub>1122</sub> 1d later Me2 <sub>1112</sub> 3d later		C&D8 <sub>1001</sub> T3 <sub>1122</sub> Q3 <sub>1001</sub> C23 <sub>1122</sub>	*C4 <sub>1122</sub>	
	2	2	1	1	4	1	
	4		2		5		11
Total.....							61

\**Pneumococcus Mucosus*.

TABLE 2.

SYNOPSIS OF CHARACTERISTICS OF PNEUMOCOCCUS CULTURES BEFORE PASSAGE THROUGH ANIMALS, AND AFTER PASSAGE THROUGH LAST SERIES.

Original Culture			Original at time of Isolation	Original January, 1906	Original before Inoculation May, 1906	Mice					Original June, 1906
						1	2	3	4	5	
*41001	{ Morphology	{ Typ.	+	+†	†	+†		+			+
		{ Atyp.	+	-	-	-	Dis-	+			-
		{ Caps. Inulin	+				carded	+	Lived		
†16112	{ Morphology	{ Typ.	†	†+	+		+	†+			†
		{ Atyp.	?	-	-	+	†	†	Dis-	+	
		{ Caps. Inulin	+			+	†	†	carded	Indic.	+
361001 (No. 1)	{ Morphology	{ Typ.	+	+	+	Dis-	+	+	+		+
		{ Atyp.	+	-	-	carded	-	-	No org		-
		{ Caps. Inulin	+						Lived		
361001 (No. 2)	{ Morphology	{ Typ.	+	+	+	Not					+
		{ Atyp.	+	+	+	inoc.					+
		{ Caps. Inulin	+								+
471001	{ Morphology	{ Typ.	+	+	†+	†+	+	†+	+	+	+
		{ Atyp.	+	-	-	-	-	-	-	No org	+
		{ Caps. Inulin	+			†		†	†		
761001	{ Morphology	{ Typ.	+	†	+	+	+	+	+	+	+
		{ Atyp.	Indic.	-	-	Indic.	-	-	-	-	-
		{ Caps. Inulin	+			+	†	†	†	†	
N231122	{ Morphology	{ Typ.	+	+†	+	+	+	†+	†	+	+
		{ Atyp.	+	-	-	?	+	-	+	+	+
		{ Caps. Inulin	+			+	+	†	†		
N911222	{ Morphology	{ Typ.	+	†	+	+	+	+			+
		{ Atyp.	+	-	-	+	+	+	Lived		+
		{ Caps. Inulin	+			+					+

\*+† = Plate typical, serum broth not characteristic.

††+ = Plate not characteristic, serum broth typical.

1001 = Organism isolated from plate.

1122 = " " " 1st rabbit.

1222 = " " " 2d "

1112 = " " " mouse.

Twenty-seven strains were studied which came originally from cases of pneumonia. Nine of these coagulated inulin not later than in original tests. Seven were fairly characteristic morphologically, two not characteristic; six coagulated later than in original tests, five characteristic, one not characteristic; while 12 did not coagulate in these cultures, though all gave a positive reaction when first tested.

When first studied at the time of isolation, 21 of the above were typical, six fairly typical.

Twenty-three strains from normal cases were studied, eight of these coagulating inulin not later than in first tests, four being characteristic, four not characteristic; two coagulated later than in first tests, both characteristic, and 13 did not coagulate at this time, although positive in original tests. When first studied 13 were typical, four not characteristic, and six atypical.

TABLE 3.  
TOTAL INULIN TESTS: 4 CASES.

CASE	STRAIN		DATE OF CULTURE					TOTAL RESULTS			TOTAL TESTS
			At Time of Iso- lation	January, 1906	March, 1906	May, 1906	June, 1906	—	±	+	
N231122	Original strain	{ — + + — — + +<									

NOTE.

Total coagulating cultures in black-face figures.

M. A.=first mouse inoculated.

M. 1.= " " " of second series.

M. M. 1.=first mouse inoculated from mouse of first series.

M. R. 1.= " " " from rabbit.

R. A.=first rabbit inoculated.

R. R.=rabbit inoculated from rabbit.

TABLE 3.—Continued.

CASE	STRAIN		DATE OF CULTURE					TOTAL RESULTS			TOTAL TESTS		
			At Time of Iso- lation	January, 1906	March, 1906	May, 1906	June, 1906	-	+	+			
No 11222	Original strain	}	3	1	2	3	6	12			}	15	
	M. A.				1							3	1
	M. B.				2							1	2
							1		1	2			8
	M. C.					1	1	3				5	
	R. A.				2							2	
	R. B.							1		1			5
					1	1	2					4	
	M1							3	3				2
	M2							2	2	2			3
								2					
	M3							4				4	8
	M. M. 1							8				8	8
								1	1				
	M. M. 2							2		2			7
								4				4	
	M. R. 1												4
												4	
M. R. 2					4				5				
					1		1						
					4			4					
Total.....								21	8	41	70		

TABLE 3.—Continued.

CASE	STRAIN		DATE OF CULTURE					TOTAL RESULTS			TOTAL TESTS
			At Time of Iso- lation	January, 1906	March, 1906	May, 1906	June, 1906	—	+	+	
41001	Original strain	{ +<									

TABLE 3.—Continued.

CASE	STRAIN	DATE OF CULTURE	MORPHOLOGY			INULIN					TOTAL TESTS
			Typical	Atypical	Capsule	Total Results					
						—	Sl.	±	+	+†	
N62 <sub>1122</sub>	Original	Mar. '05		+	—	4					4
	"	Jan. '06	±	+		1					1
	"	Mar. '06		+		1					1
	*R. A.	" '06	±	+	No org.				1		1
	R. B.	" '06		+	No org.	1					1
	M. A.	" '06		+	+	1					1
	Original	May, '06		+		1					1
	"	June, '06		+		3	1	2	2		8
	R. A.	" '06		+		2		2	1		5
	†M. R. 1	" '06	±	+	—	1	1	10	2		14
	M. R. 2	" '06		+	No org.	2		3	1		6
	M. R. 3	" '06	±	+	—	1		2	1		4
	M. R. 4	" '06		+	Indic.	1		3	1		5
	R. R.	" '06		+	—				3	1	4
	Total.....						19	2	22	12	1

\*Plate atypical; serum broth typical.

† " typical; " atypical.

Of the 11 strains from miscellaneous cases, four coagulated inulin, not later than when first studied, two characteristic, one not characteristic; and five failed to coagulate, although positive in original tests, four of these characteristic, one not characteristic. When first studied six were typical, five not characteristic.

Unless extremely small, size of organisms has not been considered in this division into characteristic and non-characteristic, the classification being based upon the general morphological picture.

In order to ascertain the effect upon the inulin fermenting property produced by passage of the organism through animals, a certain number of strains were selected for further study, and in March, and again in June, of the present year, these were inoculated into mice and rabbits.

In these experiments no attempt was made to ascertain the smallest lethal dose, but all strains were found to have lost virulence, very large doses being required to kill the animals. In the last series of mouse inoculations (Mouse 1, Mouse 2, etc.) the organism was passed directly from one animal to another; the heart being divided, the chest cavity then washed with sterile water, and this inoculated intraperitoneally, cultures being made at the same time.

By this method, a high degree of virulence was developed in some cases, mice dying within six to eight hours after inoculation, with profuse cultures of the pneumococcus given by the heart's blood.

Table 2 gives a synopsis of morphology and inulin coagulation with the original strains and after passage through a second series of animals.

Table 3 gives the complete record of inulin tests made with four strains, the results in these cases being typical of those given by the other cases studied.

Of the comparatively small number of cultures studied only two have coagulated the inulin medium in recent tests which failed to coagulate it when first isolated. These two, 16<sub>1112</sub> and N 62<sub>1122</sub>, were not included in the January table.

No. 4<sub>1001</sub> originally a large, typical pneumococcus, showing capsules and coagulating inulin, has in these tests shown a variable morphology, with loss of capsules and inulin-fermenting power, but after passage through Mouse 3 there is again a typical morphology, with capsules, and two cultures showing complete and two partial inulin coagulation. Of a total of 23 inulin tests, three are positive, two partial, and 18 negative. To this list may be added at least six coagulations not recorded, made during the first few months after isolation, when the organism was frequently tested and always gave prompt positive results. The negative tests have all been made since the beginning of January.

No. 16<sub>1112</sub> which has shown a mixed streptococcus and pneumococcus morphology throughout cultivation, and was negative to inulin when first tested, now gives positive inulin coagulation in cultures, both from the original strain and after passage through Mouse 5. Of the total inulin tests three are positive, eight partial, and 20 negative.

No. 36<sub>1001</sub> has been of special interest. It was originally a very typical pneumococcus, showing capsules, coagulating inulin promptly, and very virulent for animals. It now shows in two series of cultures from the same strain, entirely opposite characteristics. The one series consists of later transfers from a series which in the summer of 1905 was carried on for many generations on special media namely: horse blood agar, rat blood agar, rabbit blood agar, and mouse blood agar; cultures being then tested for virulence and inulin coagulation. Originally virulent for mice in doses of 1/1,000,000 c.c., rabbits 1/5,000, and rats 1/10 c.c., all cultures were now found to have decidedly lost in pathogenic power for these animals. Inulin was still promptly coagulated in seven cultures. Tried again in October of the same year, the cultures were found to be non-virulent for rabbits, in 4 c.c. doses and no longer killed mice unless given in large doses of a strong emulsion. No further tests of virulence have been made with this series. The cultures are still fairly typical morphologically, have recently coagulated inulin, and have given 12 positive coagulations during the past six months.

The contrasting series of 36<sub>1001</sub> consists of the regular laboratory stock cultures. In these a complete change has taken place, and the organism now grows as a typical streptococcus in all media, is cultivated with difficulty, and shows no reaction with inulin serum water. This change has been a gradual one, all degrees of morphology



having been observed between the two types of organisms, and repeated fishing from atypical colonies have given cultures of a similar mixed growth. Of 56 total inulin tests, 23 have been positive, 33 negative.

No. 47<sub>1001</sub> is of peculiar interest on account of its morphological changes. Originally a typical *Pneumococcus mucosus*, it has shown in subsequent cultures a varying morphology, sometimes of a typical pneumococcus and again of a streptococcus type, with occasional reversions to the original *Pneumococcus mucosus*, the latter form generally appearing only after passage through animals, especially mice, or when a fresh transfer is made after a long resting period. After persisting for a varying number of transfers (from one or two to eight) the *Pneumococcus mucosus* form is again lost, the organism reverting to the pneumococcus, the streptococcus, or to mixed types. Table 4 shows the variations observed after continued cultivation on various media, according to plan described under 36<sub>1001</sub>. In the transfers following those given in the table all the horse blood agar cultures showed the *Pneumococcus mucosus* form, which persisted for eight weekly transfers, the pneumococcus, the streptococcus, or the mixed forms then reappearing. In January last a typical *Pneumococcus mucosus* was again obtained from this series, the form persisting for several transfers.

The changes of form observed in this organism are in unison with the results previously obtained at this laboratory, and are a confirmation of the intimate connection and interchangeable nature of the pneumococcus and *Pneumococcus mucosus* types of organism.

No. 47<sub>1001</sub> was originally virulent for mice in 1/100,000 c.c., rats, 1/100 c.c. Tested after growth on the various blood agars, a loss of virulence was found in all cultures. Some difference was noticed in cultures according to the media upon which growth had been carried on, but upon the whole the results were irregular, and without further tests no definite conclusion could be drawn upon this point.

The coagulation of inulin, while at first prompt, has since been irregular with this organism. Of 54 inulin tests, 13 are positive, six partial, and 35 negative.

No. 76<sub>1001</sub> is another organism which has shown a marked change. Originally a typical pneumococcus, coagulating inulin promptly, in these tests it has appeared as a characteristic short streptococcus, and with the exception of two positive coagulations from Mouse 1, and four partial reactions from other animals, all recent inulin tests have been negative. Of 31 total inulin tests three are positive, four partial, and 24 negative.

N 62<sub>1122</sub> has shown features of peculiar interest and in the summary results have been given more in detail. With the exception of a few cultures which more nearly approach the pneumococcus type, this organism has presented the appearance of a typical streptococcus during the entire period of cultivation. It was negative to inulin when first isolated and in many subsequent tests, but in the recent series of experiments coagulations have been obtained with cultures from each animal, and also from the original strain. Of 56 inulin tests, 13 have been positive, 24 partial, and 19 negative.

N 23<sub>1122</sub> and N 91<sub>1222</sub> were both originally typical pneumococci, showing capsules, coagulating inulin promptly, and virulent for animals. With the exception of an occasional culture showing increased chain formation, both strains have been typical in morphology throughout, N 91<sub>1222</sub> especially showing the most typical large pneumococci, but the record of inulin tests shows that with both strains many cultures

have failed to coagulate inulin. After passage through animals coagulation again occurs, and recently positive inulin tests have also been obtained from the original strain of N 23. N 23<sub>1122</sub> in 63 inulin tests shows 18 positive, 22 partial, and 23 negative; N 91<sub>1222</sub> in a total of 70 tests gives 41 positive, eight partial, and 21 negative; we have therefore but 35 per cent and 58.5 per cent respectively of positive inulin tests for these two very typical strains of pneumococci.

TABLE 4.

SHOWING VARIATIONS IN MORPHOLOGY (PNEUMOCOCCUS AND PNEUMOCOCCUS MUCOSUS FORMS), AND INULIN COAGULATION OF 47<sub>1001</sub> IN AUGUST, 1906, AFTER CONTINUAL CULTIVATION ON SPECIAL MEDIA. FIRST SERIES TRANSFERRED DAILY: SECOND SERIES AFTER INTERVAL OF NINE DAYS.

SERIES	DATE OF CULTURE	MEDIUM	TOTAL TRANSFER NO.	TRANSFER ON SPECIAL MEDIUM	TYP. PNEUMOCOCCUS MUCOSUS		TYP. PNEUMOCOCCUS		INULIN COAGULATION
					Growth	Smear	Growth	Smear	
Horse Blood Agar	Transferred daily	8/9 Horse bl. agar	90	30	+	+			
		8/9 Serum broth	90			+			
		8/10 Inulin				+			
	Transferred 9th day	8/11 Bl. agar plate					+	+	+
		8/9 Horse bl. agar	85	25	+	+			
		8/9 Serum broth	85				+	+	
Rabbit Blood Agar	Transferred daily	8/9 Rabbit bl. agar	101	41	+	+			
		8/9 Serum broth	101			+			
		8/10 Inulin				+			
	Transferred 9th day	8/11 Bl. agar plate					+	+	+
		8/9 Rabbit bl. agar	96	35	+	+			
		8/9 Serum broth	96			+			
Rat Blood Agar	Transferred daily	8/9 Rat bl. agar	101	40	+	+			
		8/9 Serum broth	101			+			
		8/10 Inulin				+			
	Transferred 9th day	8/11 Bl. agar plate					+	+	+
		8/9 Rat bl. agar	96	35	+	+			
		8/9 Serum broth	96			+			
Mouse Blood Agar	Transferred daily	8/9 Mouse bl. agar	102	11	+	+			
		8/9 Serum broth	102			+			
		8/10 Inulin				+			
	Transferred 9th day	8/11 Bl. agar plate							+
		8/9 Mouse bl. agar	97	6	+	+			
		8/9 Serum broth	97			+			
		8/10 Inulin				+			
		8/11 Bl. agar plate					+	+	+

Blood agar and serum broth cultures made from blood agar of August 8, in each case. Plates made from inulin cultures.

Considered as a whole, it will be seen that the entire series of inulin tests here reported is characterized by a marked irregularity as to coagulation results, this being the case not only with atypical, but also with typical strains.

As the tables show, passage through animals seems generally to have a favorable influence upon the inulin fermenting power of the pneumococcus, an effect best shown in the strain from N 91.

The number of organisms inoculated into the inulin medium is often found to be an important factor, as coagulations have several times been obtained by the use of a strong emulsion of organisms after many negative results with cultures of ordinarily abundant growth. This, however, is not an invariable rule, as good coagulations have been obtained in some cases from cultures showing a very poor growth, while other cultures of the same inulin lot, containing a heavy growth of the same organism, have failed to coagulate.

The growth of the organisms in inulin has been studied in all cases, either in smears or by blood-agar streak plates, whenever possible, by both methods, and no cultures have been included in the report which have not been found to contain an abundant number of organisms; the large number discarded on account of insufficient growth causing the irregularity in the number of tests recorded for each strain. All inulin cultures were incubated for two weeks before being classed as negative.

The inulin serum water used in these tests was made according to the usual method, with one-third ox serum, two-thirds distilled water, and 1 per cent inulin powder, each lot being tested with laboratory stock cultures before being used in these experiments. The inulin powder used in some of the earlier work was extracted by Dr. Gibson at the Research Laboratory from dandelion roots, a preparation which gave very good results. For all the other tests the medium used was prepared from Merck's inulin (white). Various stocks of this powder, procured at different periods were found to differ greatly, and one whole stock had to be discarded, as no reliable tests could be obtained from it. In all of the work done in March and subsequently, but one stock of Merck's white inulin has been employed, 5 c.c. of the inulin serum water being inoculated with  $\frac{1}{2}$  c.c. of culture in each test. The greatest variation has been found between lots of inulin serum water made at different dates from this one inulin powder, and from the same or different lots of ox serum. Tubes inoculated with the same strain show all stages between entirely negative and positive results and an equal irregularity is found in many cases when tubes of the same or different lots of inulin medium are inoculated simultaneously with the contents of the same culture tube. Some difference was noticed between lots of inulin medium according to the supply of ox serum used, but at the end of the time-limit differences based upon this distinction were either very slight or no longer noticeable. Table 5 gives the results of the tests made with N 23122 and N 91222 in five lots of inulin medium made of the same inulin powder, and two different lots of ox serum.

TABLE 5.

RESULTS OF TESTS WITH FIVE LOTS OF INULIN SERUM WATER. ALL MADE FROM SAME INULIN POWDER (MERCK'S WHITE) FROM TWO LOTS OF OX SERUM—2 CASES.

CASE	STRAIN	TESTS GROUPED UNDER LOT OF INULIN MEDIUM USED										TOTAL TESTS		
		Tubes Inoculated						Results						
		1st Ox Ser.			2d Ox Ser.			Coagulation						
		Lot			Lot			-	SL	+	+		+	
N237112	Orig.	1	1	1	1	1		1	1			1		5
	M 1	1		1		3	1			1	1	1		6
	M 2	1		2		3	1			3	1	1	1	7
	M 3		1			1	1	1		1	1			3
	M 4		1			1	1	1		1	1			3
	M 5		1		1	2	1	1		1	1	1		5
	R. B.			1		1	3	2		1	1			5
	MR 2		1		3	3	3	1	2		1	1		10
	MR 2			1		1	1	2			1	1	1	3
	Tests Grouped According to Ox Serum used.	Total .....	..	..	..	..	..	47	13		18	12	4	47
1st Ox Serum		3	5	10				1	5	1	2	5	2	
							18	7		2	5	4	18	
2d Ox Serum					16	13		4		11	9	5	2	
							29	4		18	7		29	

TABLE 5.—Continued.

CASE	STRAIN	TESTS GROUPED UNDER LOT OF INULIN MEDIUM USED										TOTAL TESTS			
		Tubes Inoculated						Results							
		1st Ox Ser.			2d Ox Ser.			Coagulation							
		Lot			Lot			-	SL	+	+		++		
N 91222	Orig.	I	I	I	I	2		I						6	
	M 1				I	I		I						2	
	M 2		I		I	I		I						3	
	M 3		I	3	3	I		I	I		I	3	I	5	
	R. B.			I	I	I				I		I	I	3	
	MR 1		I	2	I							I	I	4	
	MR 2			3	2	2				I		2	I	7	
	MR 3			I									I	1	
	M. D.		I	2		I				I		2	I	4	
	MM 1			4	3	I						3	3	I	8
	MM 2		I	2	I	2		I				I	I	I	6
	Tests Grouped According to Ox Serum Used	Total.....	..	..	..	..	..	52	14	I	5	27	5		52
		2d Ox Serum	I	6	19				I	4	2	I	I	10	5
1st Ox Serum						14	12	26	7	I	2		11	5	26
									3		2		9		
								26	7		3		16		26

A few cultures of streptococci recently obtained from various pyogenic sources were inoculated into animals, and the morphology and inulin growth in media studied as above. One of these strains resembles a pneumococcus, and one a *Pneumococcus mucosus* in several cultures, and all show capsules after passage through animals. Table 6 gives a summary of results and shows that all inulin cultures remain negative. These experiments are too few to be of any importance, but are given as controls, and because it seems possible that further study in this direction may establish the pneumococcus nature of some organisms now classed as streptococci.

TABLE 6.  
SYNOPSIS OF CHARACTERISTICS OF STREPTOCOCCUS CULTURES BEFORE AND AFTER PASSAGE THROUGH ANIMALS.

CASE			ORIGINAL STRAIN	RABBITS	MICE					
					M. A.	M. B.	M. C.	M. D.	M. E.	M. F.
* Trachoma	{ Morphology	{ Typ.	+	+	+	+	±	±	±	±
		{ Atyp. Caps. Inulin	-	+	-	+	-	+	+	-
† Empyema	{ Morphology	{ Typ.	+	±	+	±	+	Lived		
		{ Atyp. Caps. Inulin	-	+	+	+	+			
Puerperal Septicemia	{ Morphology	{ Typ.	+	+	+					
		{ Atyp. Caps. Inulin	-	+	?	Not inoculated				

\* Many cultures suggest resemblance to *Pneumococcus mucosus*, most marked in cultures from Mouse A.

† Cultures from rabbit and from Mouse B suggest resemblance to pneumococcus.

Tests of the various lots of inulin powder and of the inulin medium before inoculation have so far led to no explanation of the varying coagulation results. No differences could be detected between the several stocks of inulin powder, the rejected stock appearing to be identical with the rest except that coagulations could not be obtained with the medium made from it. Nine lots of the inulin medium made at different dates from good powder were tested for acidity before inoculation with organisms. Five c.c. titrated with N/50 NaOH and phenolphthalein were found to vary between neutral and 1.4 acid, the majority being about 0.2 acid.

Flasks containing 50 c.c. of inulin medium after titration for initial acidity were inoculated each with 5 c.c. of one of the cultures of the strains studied, and inoculated for 10 days. Some of the contents of these flasks was pipetted out every one or two days and tests made of reactions and growth in the inulin medium. Two flasks inoculated with N 91<sub>1222</sub> showed coagulation nearly complete in 24 hours, with acidity 2.2 and 2.8 respectively, organisms very characteristic, and no further tests possible because of coagulation. All flasks inoculated with other organisms gave a negative coagulation result, and showed practically no increase in acidity, while at the same time the count of plate colonies showed a good growth of organisms present. Table 7 gives the results of these tests with four of the previously mentioned strains and also with one strain of streptococci; the highest acid production being given in each case compared with the reaction before inoculation, and with the highest plate count.

TABLE 7.  
RESULTS OF TITRATION AND PLATE COUNT WITH NON-COAGULATION OF INULIN FLASK CULTURES—  
5 C.C. TITRATED WITH N/50 NaOH.

STRAIN	TITRATION		PLATES
	Before Inoculation	Highest Acidity	Highest Count to 1 c.c.
16 <sub>1112</sub> . . . . .	1.4	1.9	98,102,440
47 <sub>1001</sub> . . . . .	1.1	1.7	128,142,000
N23 <sub>1122</sub> . . . . .	0.4	0.9	45,806,050
N62 <sub>1122</sub> . . . . .	0.2	1.7	73,710,000
Streptococci . . . . .	0.2	1.8	33,015,000

Further work in this direction was contemplated but has not yet been carried out, the same being true of plans to attempt to ascertain the nature of the acid produced by the growth of the pneumococcus in the inulin medium.

#### CONCLUSIONS.

Many strains of pneumococci, after longer or shorter periods of cultivation on artificial media, are found to undergo decided changes in morphology, virulence, and power to ferment inulin.

These changes may be temporary, disappearing when the organisms are placed under favorable conditions, but in some cases they seem to be permanent, the organisms having apparently undergone a complete change from their original characteristics.

The change in morphology is toward a more or less complete approach to a streptococcus type, some tendency in this direction appearing in certain cultures of every organism studied.

The presence of the pneumococcus and of the *Pneumococcus mucosus* types in organisms of the same strain shows the close relationship and interchangeable character of these two types.

The change in virulence corresponds with that usually found after long artificial cultivation. By transfers directly from animal to animal a rapid increase of virulence is developed.

The change in inulin fermenting power represents a marked irregularity of reaction constituting the chief feature in this series of tests which, including those made at time of isolation, consists of 452 inulin tests made with cultures from 63 strains of organisms.

A large number of negative tests have been given by very typical pneumococci and, on the other hand, many coagulations have been obtained from cultures of a definite streptococcus type.

Great variations have been found between different stocks of inulin powder and also between different lots of inulin medium made from the same powder and inoculated with organisms of the same strain.

Passage through animals seems, in many cases, to have a favorable effect upon the inulin fermenting power of the pneumococcus.

The use of strong emulsions of organisms sometimes produces coagulation, where ordinarily abundant cultures give only negative results.

The conclusion drawn from these experiments is that while coagulation of inulin is thought to be valuable corroborative evidence in favor of the pneumococcus nature of an organism, yet the irregular nature of the reaction may make it a fruitful source of differences and errors in diagnosis if too much reliance is placed upon this test, since it is evident that no organism can be rejected as a pneumococcus because of one, or even of several, non-coagulating inulin cultures. Especially is this true of cultures which have been grown for some time on artificial culture media.